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Case-Control Study of Cancer among US Army Veterans Exposed to Simian Virus 40-contaminated Adenovirus Vaccine

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Simian virus 40 (SV40) was an accidental contaminant of vaccines produced in monkey kidney tissue cultures in the 1950s and early 1960s, including a parenteral adenovirus vaccine given to several hundred thousand US military recruits. Detection of SV40 DNA in tumor tissues by some laboratories suggests that SV40 contributes to human cancers. To determine if entry into US Army service during periods of administration of SV40-contaminated adenovirus vaccine was associated with an increased risk of cancer, the authors conducted a case-control study of cancer occurring in male Army veterans who entered service in 1959–1961. Cases of brain tumors (n = 181), mesothelioma (n = 10), and non-Hodgkin's lymphoma (n = 220) were identified through a Veterans Administration hospital discharge database, as were colon cancer and lung cancer controls (n = 221). Exposure to adenovirus vaccine was assigned on the basis of known periods of adenovirus vaccine administration and dates of Army entry obtained for cancer cases and controls. The odds ratios associated with exposure to SV40-contaminated adenovirus vaccine were 0.81 (95% confidence interval (CI): 0.52, 1.24) for brain tumors, 1.41 (95% CI: 0.39, 5.15) for mesothelioma, and 0.97 (95% CI: 0.65, 1.44) for non-Hodgkin's lymphoma. These findings do not support a role for SV40 in the development of these cancers.

brain neoplasms; lymphoma, non-Hodgkin; mesothelioma; military personnel; simian virus 40; United States Department of Veterans Affairs

Abbreviations: BIRLS, Beneficiary Identification and Records Locator Subsystem; CI, confidence interval; ICD, *International Classification of Diseases*; OR, odds ratio; PTF, Patient Treatment File; SV40, simian virus 40.

Simian virus 40 (SV40), a macaque polyomavirus, was an accidental contaminant in vaccines produced from monkey kidney tissue cultures in the 1950s and early 1960s. SV40 was present in the inactivated poliovirus vaccine, which was administered to 10–30 million people, mostly children, in the United States from 1955 to 1961 (1). SV40 was also present in other vaccines, notably adenovirus vaccines given parenterally to several hundred thousand US military recruits (2).

These human exposures to SV40 raise concern, because several lines of laboratory evidence suggest that SV40 could play a role in the etiology of some human malignancies. SV40 codes for T antigen, a nonstructural protein that can bind to and inhibit tumor suppressor proteins (e.g., M_r 53,000 protein (p53) and retinoblastoma protein (pRb)) (see reviews (3, 4)). In experimental rodents, SV40 inoculation leads to the development of various tumors, in particular mesothelioma (5), ependymoma (6, 7), osteosarcoma (8, 9), and leukemia and lymphoma (9). More directly, some laboratory studies have reported the detection of SV40 DNA sequences in a variety of human tumors, including mesothelioma (10, 11), brain tumors (especially ependymoma and choroid plexus tumors) (12), and non-Hodgkin's lymphoma

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(13, 14). In contrast, other laboratory studies have not confirmed these findings (15–20).

Retrospective cohort studies have not identified increased cancer risk in recipients of SV40-contaminated poliovirus vaccines (see review (21)). A potential limitation of US-based studies has been that only 10–30 percent of lots of poliovirus vaccine in the United States was actually contaminated with live SV40 (1), so assigning SV40 exposure status on the basis of receipt of vaccine could conceivably have prevented researchers from identifying an effect. Additionally, prior retrospective studies of children exposed to SV40-contaminated poliovirus vaccines had little opportunity to detect an increased risk for cancers (such as mesothelioma) that arise primarily in middle- or older-aged adults, given the relatively young ages attained during follow-up.

Beginning in 1960, the US Army routinely vaccinated recruits against adenovirus to prevent epidemics of acute respiratory illness during basic training (2). As we review below, there is compelling evidence that this vaccine was widely contaminated with SV40. To better understand the potential cancer risk associated with SV40 infection, we conducted a case-control study of cancers occurring in male US Army veterans who entered Army service between January 1959 and December 1961, some of whom received adenovirus vaccine. Cases of brain tumors, mesothelioma, and non-Hodgkin's lymphoma were identified through a Veterans Administration (later the Department of Veterans Affairs) hospital discharge database, as were a random sample of colon cancer and lung cancer controls. Dates of Army entry were obtained for cancer cases and cancer controls, and exposure to adenovirus vaccine was assigned on the basis of known periods of administration of the vaccine.

MATERIALS AND METHODS

Production, contamination with SV40, and administration of adenovirus vaccine

In the 1950s, the US Army developed an inactivated vaccine against adenovirus types 4 and 7 (2). In production of the vaccine, adenovirus was propagated in kidney tissue from macaques, many of whom were infected with SV40 (22, 23). This resulted in frequent SV40 contamination of tissue culture systems (22, 23). Furthermore, it is now known that adenoviruses grow extremely poorly in monkey kidney tissue unless SV40 is also present (24). Because SV40 greatly facilitates growth of adenoviruses, it is considered highly likely that, through strong positive selection in the laboratory, nearly all adenovirus seeds and the vaccine pools grown from them in this era quickly became contaminated with SV40 (23, 25). This situation is unlike poliovirus vaccine contamination, which did not occur uniformly, because SV40 is not a necessary cofactor for poliovirus replication in vitro.

In the final step of vaccine manufacture, vaccine pools were treated with formalin to inactivate live adenovirus. However, SV40 is relatively resistant to formalin inactivation. It is unknown how much live SV40 remained in the final adenovirus vaccine product, which would have varied some with manufacturing technique. However, because inactivation techniques were similar for adenovirus and poliovirus vaccines, it is likely that the amount of live SV40 was similar to that which was present, albeit more variably, in the inactivated poliovirus vaccine. Of importance, in 1961, Gerber et al. tested three samples of formalin-inactivated adenovirus vaccine "prepared by [a] domestic manufacturer" (26, p.206), and all samples had live SV40. Furthermore, in a 1956 adenovirus vaccine trial conducted at Fort Dix, New Jersey, 100 percent of evaluated subjects (nine of nine subjects) demonstrated seroconversion to SV40 (23). Thus, the positive selective pressure driving widespread SV40 contamination of adenovirus vaccine pools, the detection of live SV40 in formalin-inactivated vaccine, and the documented seroconversions following vaccination together point to frequent contamination of this vaccine.

The US Army began routine use of this parenteral adenovirus vaccine in 1960. Vaccination was administered on the first day of basic training, corresponding closely with the recorded date of entry into service. According to a 1961 article by the chief of the US Army's Communicable Disease Branch, Office of the US Army Surgeon General, adenovirus vaccine was given universally to all recruits entering Army service from February through April of 1960, but "production difficulties" (2, p. 1126) then interrupted vaccination until August 1960, when vaccination resumed. Recruits were again routinely vaccinated from August 1960 through May 1961, when vaccination ceased because "supplies once more became limited" (2, p. 1126). Other evidence suggests that adenovirus vaccine was withdrawn in 1961 because of concerns regarding contamination with SV40. Specifically, a 1994 history of the Armed Forces Epidemiology Board states that "SV40 was found to be present in the killed adenovirus vaccine as well as in viral seed stocks…. In consequence, the Division of Biologics, National Institutes of Health, acted in 1961 to prevent the distribution of vaccines containing viable SV40 virus" (27, p. 38).

Persons entering the Army in 1959–1961 also had exposures to inactivated poliovirus vaccine possibly contaminated with SV40. For instance, in 1961, 84 percent of US residents aged 15–19 years had previously received one or more doses of inactivated poliovirus vaccine (28). Similar estimates were reported specifically for US military recruits in 1962 (29). Because of concerns regarding the risk of developing poliomyelitis during service, beginning in 1959, the Army required all entering personnel to have received the basic series of three poliovirus vaccinations plus a single booster. However, only a minority of inactivated poliovirus vaccine doses contained live SV40 (1). Thus, the defined periods of uniform exposure to SV40 through adenovirus vaccination still provided an opportunity to study the long-term effects of SV40 exposure on cancer risk.

Study population and ascertainment of cases and controls

We conducted a case-control study with cancer controls (30). Cases and controls were restricted to men who entered US Army service between January 1959 and December 1961

at the ages of 17–30 years and who were treated for cancer within the Veterans Administration medical system. The case-defining conditions were brain tumors, mesothelioma, and non-Hodgkin's lymphoma, each considered as possibly related to SV40. We used as controls patients treated for other cancers (rather than noncancerous conditions), since Veterans Administration referral patterns across cancer types would be somewhat similar. Specifically, controls were selected from patients treated for lung cancer or colon cancer, two types of cancer believed to be unrelated to SV40. Women represented less than 1 percent of Army personnel entering in this era and were excluded from this study.

Cancer diagnoses for both cases and controls were ascertained using the Patient Treatment File (PTF) maintained by the Veterans Administration. The PTF file is a computerized record of hospital discharge diagnoses for all hospital visits covered by the Veterans Administration, and the file provided data for 1969–1996. To ensure uniform PTF ascertainment of cancers in men who entered the Army across the 1959–1961 period, we further required that cancers arise in the period 10–35 years after Army entry (i.e., in 1969–1994 for men entering in 1959 and in 1971–1996 for men entering in 1961).

Notably, the PTF does not have information on the branch of service or date of entry into service, so this information was obtained by linking to another database (see "Matching and exposure assessment" below). Thus, we first identified potential cases and controls as individuals with cancer who might have entered the Army during the period of interest. To do this, we restricted consideration to the PTF diagnoses in males born between January 2, 1928, and December 31, 1944, that is, the only range of birth dates that corresponded to possible entry into the Army in 1959–1961 at ages 17–30 years. We selected all such men with PTF diagnoses of brain tumor (*International Classification of Diseases* (ICD), Revision 8 or 9, code 191), mesothelioma (ICD code 163), and non-Hodgkin's lymphoma (ICD codes 200 and 202) as potential cases. To maximize feasibility of matching and exposure assessment for potential controls, which were much more common than cases, we randomly selected 5,000 men with colon cancer (ICD code 153) and 5,000 men with lung cancer (ICD code 162).

The study protocol was approved by the institutional review boards at the National Cancer Institute, the National Academies, and the Johns Hopkins Bloomberg School of Public Health.

Matching and exposure assessment

The identifying information provided by the PTF on potential cases and controls included the name, date of birth, race, and Social Security number. To obtain service branch and date of entry into service, we linked the PTF with a second database, the Beneficiary Identification and Records Locator Subsystem (BIRLS), which contains demographic and military service information for individuals who have filed a claim for a veterans' benefit (including a death benefit). Potential cases and controls identified through the PTF were linked to the BIRLS by Social Security number. Matches on Social Security number were verified by a manual review of names. As described above, included cases and controls were then defined as male veterans who entered the Army in 1959–1961 at the ages of 17–31 years and were diagnosed with cancer 10–35 years after Army entry. Among the cancer cases and controls that fit these criteria, six men were diagnosed with two cancers. Medical records were available for three of these six men who were included in the validation substudy described below, and the appropriate cancer diagnosis was assigned to these individuals based on record review. Medical records could not be obtained for the other three individuals; thus, all three were excluded from subsequent analyses.

Using the exact date of entry into service and the data provided by Sherwood et al. (2) on Army use of adenovirus vaccine, we assigned adenovirus vaccine exposure to the cases and controls. Cases and controls were considered to have been exposed if they entered during the time periods in which adenovirus vaccine was administered to all Army recruits: from February 1960 to April 1960 and from August 1960 to May 1961. The three months between these two periods (from May 1960 to July 1960) represent the period during which adenovirus vaccine was unavailable because of supply shortage. Men who entered the Army during this brief time period were designated as unexposed. Similarly, men were considered unexposed if they entered prior to the introduction of adenovirus vaccine (from January 1959 to January 1960) or immediately after the cessation of the adenovirus vaccination program (from June 1961 to December 1961). Additionally, we estimated the total numbers of men who were exposed or unexposed in the underlying cohort of 620,000 Army servicemen, using monthly Army data on the number of individuals completing basic training in 1959–1961.

Validation substudies

We conducted two separate substudies to validate both the cancer diagnoses and the exposure data (dates of Army service). First, to assess the validity of cancer diagnoses ascertained through the PTF database, we submitted a list of deceased study subjects with diagnoses of brain tumors or mesothelioma to the Veterans Administration, requesting copies of pathology reports and relevant discharge summaries. Medical records were available for 76 (42 percent) of 180 such cases, including 72 of 168 brain tumors (43 percent) and four of 12 mesotheliomas (33 percent). Of the 76 cases for which records were available, the PTF cancer diagnoses matched the Veterans Administration medical records in 63 cases (83 percent), including 61 of 72 brain tumors (85 percent) and two of four mesotheliomas (50 percent).

Second, we obtained hard copies of service charts to validate the dates of Army entry recorded in the BIRLS and to verify the correspondence of these dates to the start of basic training (i.e., vaccination). Charts were obtained for a sample of 200 men who, according to the BIRLS, entered the Army in 1959–1961 and for 100 men who entered in 1957–1958 or 1962–1963. Records were located for 268 men (89 percent), of whom 254 (95 percent) had Army service verified. The date of entry was available for 266 men (99

percent). The service chart date of entry matched the BIRLS date for 246 of these men (92 percent). For the remaining 20 men, the median difference in dates was 12.5 days; in each discrepancy, the difference between the two dates did not result in a misclassification of adenovirus vaccine exposure. Basic training start dates, available for 254 men (85 percent), followed the start of Army service by a median of 8 days (interquartile range: 4–13 days). By the date of Army entry, there was no systematic variation in verification of service branch, differences between BIRLS and service chart entry dates, or time from service entry until basic training.

Statistical methods

We calculated the proportion of men who entered the Army during a period of adenovirus vaccination for both cancer cases (brain tumors, mesothelioma, non-Hodgkin's lymphoma) and controls (lung cancer, colon cancer). To determine the effect of exposure to SV40-contaminated vaccine on the risk of brain tumors, mesothelioma, and non-Hodgkin's lymphoma, we calculated an odds ratio for each cancer site as the ratio of the odds of exposure in the cancer cases to the odds of exposure in the cancer controls (colon cancer and lung cancer combined). Brain cancer analyses were stratified by subtype when available, using histologic diagnoses obtained in our validation substudy. Odds ratios were adjusted for age at diagnosis and race using logistic regression. Under the assumptions that SV40 is not associated with colon cancer or lung cancer and that ascertainment of cancers does not differ across the cancer sites, this approach produces a valid estimate of the effect of SV40 on risk of brain tumors, mesothelioma, and non-Hodgkin's lymphoma (30). To test whether the prevalence of adenovirus vaccine exposure in controls reflected that in the underlying population, we compared the distribution of dates of Army entry among lung cancer and colon cancer controls with the distribution among the underlying cohort of 620,000 servicemen using a χ^2 test.

Confidence intervals were calculated for odds ratios, derived using an exact method when the expected counts were less than five. All statistical tests were two sided, and p values of less than 0.05 were considered statistically significant. Statistical analyses were conducted using SAS version 8.0 software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

A total of 12,298 potential cases (diagnoses of brain tumor, mesothelioma, non-Hodgkin's lymphoma) and 10,000 potential controls (diagnoses of colon cancer, lung cancer) were ascertained through PTF records. Over 91 percent of potential cases and controls were successfully matched to the BIRLS on Social Security number (figure 1). Names of patients were identical or very similar between the two databases. Almost half of the matched potential cases (n = 5,724) and controls (n = 4,995) entered the Army, of whom 482 (8 percent) potential cases and 251 (5 percent) controls entered in 1959–1961. Over 97 percent of these potential cases (n = 470) and controls (n = 249) were 17–31 years of age on the date of entry, and approximately 90 percent were diagnosed in the period 10–35 years after entry. Six cases and three controls with multiple cancers were excluded, yielding a final sample size of 411 cancer cases and 221 controls (figure 1).

There were 181 cases of brain tumors, 10 cases of mesothelioma, and 220 cases of non-Hodgkin's lymphoma identified. We included as controls 107 colon cancers and 114 lung cancers. There were significant differences in race by cancer type, with higher percentages of Whites among brain tumor, mesothelioma, and non-Hodgkin's lymphoma cases than among colon cancer and lung cancer controls (table 1). The ages at Army entry were similar for those with each cancer type (table 1). The age at diagnosis was significantly younger for all cancer cases combined than for colon cancer and lung cancer controls (mean age: 46.5 vs. 49.4 years; $p < 0.0001$).

Table 2 presents the distributions of dates of Army entry and corresponding exposure to adenovirus vaccine for cases and controls. The prevalence of adenovirus vaccine exposure was similar in cases and controls. Specifically, the odds ratios for vaccine exposure were 0.81 (95 percent confidence interval (CI): 0.52, 1.24) for brain tumors, 1.41 (95 percent CI: 0.39, 5.15) for mesothelioma, and 0.97 (95 percent CI: 0.65, 1.44) for non-Hodgkin's lymphoma. These odds ratios were not affected by adjustment for age at diagnosis and race (table 2). Additionally, as shown in table 2, the dates of entry into Army service of controls were similar to those of the underlying cohort of 620,000 men ($\chi^2_{4df} = 2.03$; $p = 0.73$).

There was no apparent association between adenovirus vaccine exposure and risk for specific subtypes of brain tumors identified through review of Veterans Administration medical files. Specifically, compared with the colon cancer and lung cancer controls, exposure to adenovirus vaccine was not positively associated with glioblastoma multiforme (odds ratio (OR) = 0.21, 95 percent CI: 0.06, 0.72; 28 cases), astrocytoma (OR = 0.56, 95 percent CI: 0.17, 1.87; 17 cases), other/unclassified glioma (OR = 0.54, 95 percent CI: 0.10, 2.86; nine cases), or miscellaneous primary brain tumors (OR = 0.83, 95 percent CI: 0.14, 4.86; seven cases). We identified only one case each of ependymoma (exposed to adenovirus vaccine) and choroid plexus tumor (unexposed).

To investigate the possibility that our assignments of vaccine exposures were off by 1 or 2 months, we lagged the cutpoints used to define exposure to adenovirus vaccine by 1 and 2 months and observed no appreciable change in odds ratios (data not shown). To accommodate the possibility that exposure was incorrectly assigned for the relatively small group of men who entered the Army from May 1960 to July 1960, the time period corresponding to the brief interruption in adenovirus vaccination, we conducted analyses excluding this group, and similar results were obtained (data not shown).

DISCUSSION

In our case-control study of cancers occurring among men who entered the US Army in 1959–1961, we found no evidence that exposure to adenovirus vaccine was related to an increased risk for brain tumors, mesothelioma, or non-

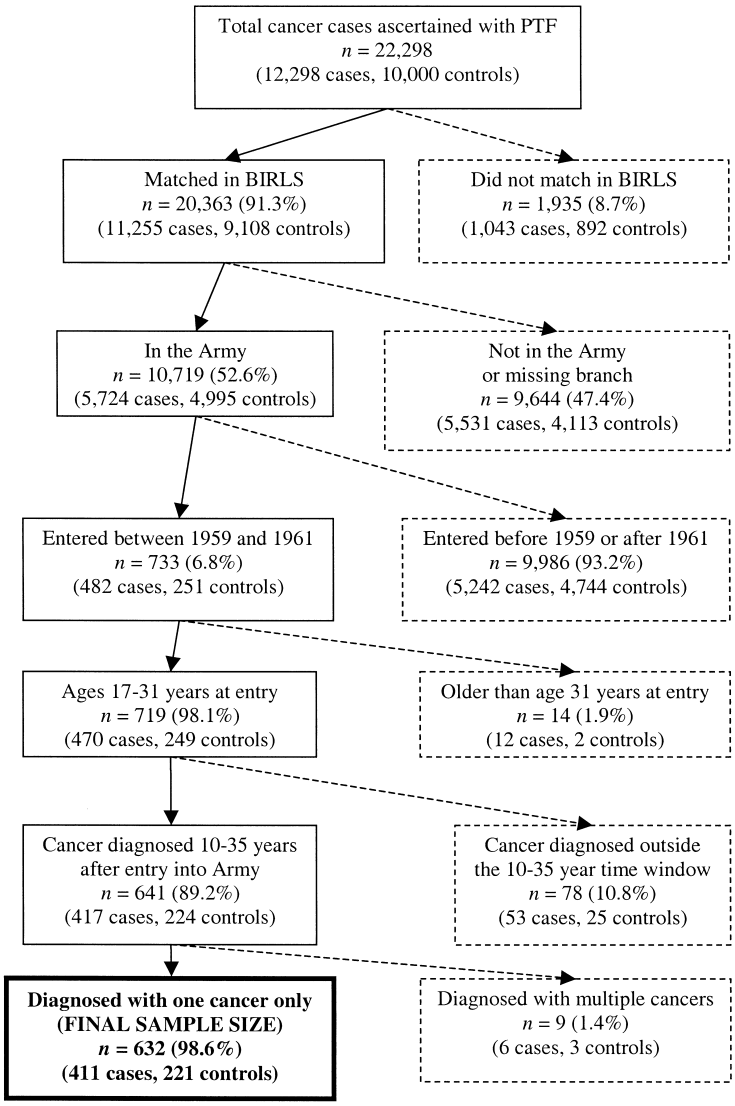

FIGURE 1. Derivation of the final study sample of US Army veterans with cancer, who entered Army service in 1959–1961. Potential cancer cases (brain tumors, mesothelioma, non-Hodgkin's lymphoma) and cancer controls (lung cancer, colon cancer) ascertained through the Veterans Administration's Patient Treatment File (PTF) database were linked to the Veterans Administration's Beneficiary Identification and Records Locator Subsystem (BIRLS) database to obtain the date of entry into military service. Those cases and controls that could not be linked to BIRLS were excluded. Additionally, potential cases and controls were excluded if they did not serve in the US Army, entered service before 1959 or after 1961, were aged more than 31 years at Army entry, or were diagnosed with cancer less than 10 years or more than 35 years after Army entry. A total of 411 cases and 221 controls fit all inclusion criteria.

Hodgkin's lymphoma. The numbers of cases of mesothelioma and specific brain cancer subtypes were small, limiting our inferences about these sites. However, the numbers of all brain tumors and non-Hodgkin's lymphoma cases were substantial, and the 95 percent confidence intervals for the corresponding odds ratios excluded an effect of large magnitude (table 2). As we review, substantial evidence indicates that the Army's adenovirus vaccine was widely contaminated with SV40. We conclude that our study provides evidence against SV40 as a cause of these cancers.

This retrospective case-control study is the first investigation of cancer risk associated with receipt of the Army's early adenovirus vaccine. Our study has two important strengths. First, exposure assignment in our study was based on specific information about when individual subjects entered the Army and published data on the Army's use of adenovirus vaccine. Given the documented contamination of this vaccine with SV40, we were able to assign SV40 exposure to subjects with reasonable confidence. Second, we captured cancers diagnosed up to 35 years after entry into service, when men had reached an age in their fifties or

TABLE 1. Characteristics of cancer cases and controls among US Army veterans, 1969–1996

Cancer type	Total (no.)	Race (%)*			Mean age at Army entry (years)	Mean age at diagnosis† (years)	Mean years from Army entry to diagnosis†
		White	Black	Other/ unknown			
Cases							
Brain tumors	181	87.9	10.5	1.7	21.0 (2.7)‡	44.9 (6.6)	23.9 (6.4)
Mesothelioma	10	70.0	20.0	10.0	20.1 (3.6)	46.9 (6.9)	26.8 (5.7)
Non-Hodgkin's lymphoma	220	80.5	18.2	1.4	20.8 (2.6)	47.8 (6.7)	27.1 (6.4)
Controls							
Colon cancer	107	71.0	28.0	1.0	21.5 (2.7)	49.0 (6.6)	27.4 (5.9)
Lung cancer	114	71.9	28.1	0.0	20.9 (2.7)	49.8 (5.6)	28.9 (5.4)

* Race differed significantly across cancer types ($\chi^2_{8df} = 27.4$; $p = 0.0006$). The proportion of Whites compared with Blacks and others was higher among brain tumor, mesothelioma, and non-Hodgkin's lymphoma cases than among colon cancer and lung cancer controls ($\chi^2_{4df} = 17.3$; $p = 0.002$).

† Both age at cancer diagnosis and years from Army entry to diagnosis differed significantly across cancer types (analysis of variance: $p < 0.0001$).

‡ Numbers in parentheses, standard deviation.

sixties. At this age, mesothelioma and non-Hodgkin's lymphoma incidence begin to increase, which facilitated our evaluation of the role of SV40 in these malignancies.

Several additional points should be considered when interpreting our data. We relied on Veterans Administration records to identify cancer cases and controls, but only an estimated 40 percent of veterans eligible for Veterans Administration care are actually patients in the Veterans Administration system (31). Although Veterans Administration patients are not representative of all Army veterans (31), it is unlikely that utilization of the Veterans Administration for medical care or identification in the PTF differed by exposure to adenovirus vaccine, which was defined by five alternating calendar time periods over a very short (i.e., 3-year) interval. Furthermore, we explicitly tested the possibility that ascertainment bias might have affected our results by comparing the distribution of these periods of alternating exposure among colon cancer and lung cancer controls with that of the underlying cohort of 620,000 Army servicemen and observed no appreciable difference. Thus, we do not believe that ascertainment of cancers through the PTF introduced bias.

TABLE 2. Distribution of cancer cases and controls by date of entry (1959–1961) into the US Army and potential exposure to adenovirus vaccine

Date of entry into Army service	Exposure to adenovirus vaccine	Total men in the underlying cohort		Cases						Controls			
				Brain tumors		Mesothelioma		Non-Hodgkin's lymphoma		Colon cancer		Lung cancer	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
January 1959–January 1960	Unexposed	219,000	35.3	58	32.0	3	30.0	59	26.8	45	42.1	39	34.2
February 1960–April 1960	Exposed	41,000	6.6	15	8.3	2	20.0	12	5.5	10	9.3	5	4.4
May 1960–July 1960	Unexposed	41,000	6.6	11	6.1	0	0.0	18	8.2	3	2.8	7	6.1
August 1960–May 1961	Exposed	164,000	26.5	35	19.3	2	20.0	57	25.9	28	26.2	28	24.6
June 1961–December 1961	Unexposed	155,000	25.0	62	34.3	3	30.0	74	33.6	21	19.6	35	30.7
	Combined												
	Exposed	205,000	33.1	50	27.6	4	40.0	69	31.4	38	35.5	33	29.1
	Unexposed	415,000	66.9	131	72.4	6	60.0	151	68.6	69	64.5	81	70.9
Crude odds ratio*				0.81 (0.52, 1.24)†		1.41 (0.39, 5.15)		0.97 (0.65, 1.44)		1.00 (referent)			
Adjusted odds ratio*,‡				0.76 (0.48, 1.20)		1.49 (0.38, 5.88)		0.98 (0.65, 1.47)		1.00 (referent)			

* The ratio of the odds of entering the Army during a period of adenovirus vaccination in the cases of the site hypothesized to be associated with simian virus 40 (brain tumors, mesothelioma, non-Hodgkin's lymphoma) to the odds of adenovirus vaccination in lung cancer and colon cancer cases combined, as described in the text.

† Numbers in parentheses, 95% confidence interval.

‡ Adjusted for age at diagnosis (10-year categories) and race (White vs. Black or other).

Misinformation recorded in the PTF or the BIRLS could have resulted in misclassification of case or control status or exposure, biasing our results toward the null. In our effort to validate cancer diagnoses, we were able to obtain Veterans Administration medical records for only a minority of cases, including only four mesotheliomas. Nonetheless, this substudy confirmed the cancer diagnoses in most subjects with available records. Importantly, the proportion of cancer diagnoses verified by Veterans Administration records did not differ by adenovirus vaccine exposure (data not shown). Our validation substudy of adenovirus vaccine exposure found that there was minimal error in the recording of Army entry dates in the BIRLS. Additionally, risk estimates based on varying cutpoints of Army entry dates did not indicate systematic errors in the assignment of vaccine exposure.

We also considered the possibility that exposure to poliovirus vaccine could have affected our ability to observe an association between adenovirus vaccination and cancer. As noted above, all men in our study were likely inoculated with potentially SV40-contaminated poliovirus vaccine. However, given the Army's policy regarding poliovirus vaccination, receipt of poliovirus vaccine was uniform across the 1959–1961 period and therefore would not have differentially affected those exposed or unexposed to adenovirus vaccine. Additionally, only a minority of the inactivated poliovirus vaccine doses contained SV40, and not all exposures to SV40-contaminated poliovirus vaccine would have led to infection. Thus, the added exposures to SV40 from the contaminated adenovirus vaccine should have led to observable increases in cancer risk at the hypothesized sites, if SV40 infection truly increases cancer risk.

Our negative results and those from prior retrospective cohort studies of poliovirus vaccine recipients (32–36) conflict with laboratory reports of the presence of SV40 DNA sequences in human tumors, including mesothelioma, brain tumors, and non-Hodgkin's lymphoma (8, 10–14). Nonetheless, interpretation of the results of these laboratory studies is not straightforward (37, 38). The diversity of tumors in which SV40 DNA has been found and the low levels of SV40 DNA that are detected in these tumors raise the possibility that some findings could be artifactual (37, 39). SV40 DNA sequences are present in over 200 cloning vectors used by laboratories worldwide (40), which might conceivably lead to contamination of tumor tissues during laboratory evaluation. Many laboratories have not included appropriate normal tissues as negative controls and have not incorporated masking in their evaluation of tumor specimens (15, 41). In other studies, SV40 DNA was not detected in human tumors or was detected infrequently (15–19, 42–44), and SV40 DNA sequences were not reproducibly identified in a multilaboratory study in which mesothelioma specimens were evaluated blindly (20). Finally, case-control studies utilizing serologic assays for SV40 infection have identified only low levels of SV40 antibody reactivity, suggesting that SV40 is an uncommon human infection, and have failed to detect an association between SV40 serostatus and cancer (19, 45–47).

In summary, we did not find an association between exposure to SV40-contaminated adenovirus vaccine among Army service personnel and risk of brain tumors, mesothelioma, or non-Hodgkin's lymphoma. With the recent development of sensitive and specific serologic assays for SV40 infection (48), future epidemiologic studies will need to reexamine the possible association between SV40 infection and cancer. Laboratory-based investigations utilizing molecular and serologic techniques must be based on rigorous study designs, incorporating the inclusion of appropriate controls and masking. While our results point away from SV40 as a cause of cancer, additional useful information may be gleaned from such studies.

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